Applicant: Anthony J.F. D'Apic

Serial No.: 08/984,900

: December 4, 1997 Filed

Page

The claims now in this application read as follows.

5. 1. (Thrice amended) A purified and isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of (1) nucleotides 90 -1203 of the porcine nucleic acid sequence with SEO ID NO: 7, (2) a sequence encoding a porcine polypeptide having  $\alpha$ -1.3 galactosyltransferase activity and having the amino acid sequence of SEQ ID NO:10, (3) a sequence that encodes a second polypeptide identical to said porcine polypeptide except for one or more conservative amino acid substitutions, wherein said second polypeptide retains a functional α-1,3 galactosyltransferase catalytic site, a functional membrane anchor domain and a functional stem region, and (4) a sequence complementary to the sequence of (1), (2) or (3).

Docket No.: 06868-005002

- 2. (Amended) A host cell that is transformed with a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of (1) nucleotides 90 -1203 of the porcine nucleic acid sequence with SEQ ID NO: 7, (2) a sequence corresponding to the sequence of (1) within the scope of the degeneracy of the genetic code, (3) a sequence that encodes a porcine polypeptide having  $\alpha$ -1.3 galactosyltransferase activity and that hybridizes with a sequence complementary to the sequence of (1) or (2) after a wash at 65°C in a buffer containing 0.1% SDS and SSC at a concentration not greater than 0.5 x, and (4) a sequence complementary to the sequence of (1), (2) or (3).
- 3. (Amended) A porcine  $\alpha$ -1,3 galactosyltransferase encoded by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of (1) nucleotides 90 -1203 of the porcine nucleic acid sequence with SEQ ID NO: 7, (2) a sequence corresponding to the sequence of (1) within the scope of the degeneracy of the genetic code, (3) a sequence that encodes a porcine polypeptide having  $\alpha$ -1,3 galactosyltransferase activity and that hybridizes with a sequence complementary to the sequence of (1) or (2) after a wash at 65°C in a buffer containing 0.1% SDS and SSC at a concentration not greater than 0.5 x, and (4) a sequence complementary to the sequence of (1), (2) or (3).

Applicant Anthony J.F. D'Apic al.

Serial No.

08/984,900

Filed

December 4, 1997

Page

3

46. (Twice amended) A DNA construct comprising a disrupted porcine  $\alpha$ -1,3 galactosyltransferase gene, wherein the disruption is by insertion of an exogenous sequence into said gene such that the disruption prevents expression of functional  $\alpha$ -1,3 galactosyltransferase.

Docket No.: 06868-005002

- 47. The DNA construct of claim 46, wherein said disruption is within exon 4, exon 7, exon 8, or exon 9 of the porcine  $\alpha$ -1.3 galactosyltransferase gene.
- 48. The DNA construct of claim 46, wherein said exogenous sequence is a selectable marker.
- 49. The DNA construct of claim 48, wherein said selectable marker is selected from the group consisting of the neo<sup>R</sup> gene and the hyg<sup>R</sup> gene.
- 50. The DNA construct of claim 46, wherein said exogenous sequence is flanked at its 5' and 3' ends by FRT DNA elements, and wherein stop codons have been inserted 3' to the selectable marker for each of the three reading frames for the porcine  $\alpha$ -1,3 galactosyltransferase gene.
- 51. A method for generating a porcine cell comprising at least one inactivated  $\alpha$ -1,3 galactosyltransferase gene, the method comprising:
  - (a) providing a plurality of porcine cells;
  - (b) introducing into said cells the DNA construct of claim 46;
- (c) incubating said cells such that homologous recombination occurs between the chromosomal sequence encoding  $\alpha$ -1,3 galactosyltransferase and the introduced DNA construct comprising the disrupted  $\alpha$ -1,3 galactosyltransferase gene; and
- (d) identifying a porcine cell comprising at least one inactivated  $\alpha$ -1,3 galactosyltransferase gene.
- 67. A porcine cell comprising at least one disrupted  $\alpha$ -1,3 galactosyltransferase gene, wherein the disruption is by insertion of an exogenous sequence into said gene such that the disruption prevents expression of functional  $\alpha$ -1,3 galactosyltransferase and wherein the gene,

Applicant: Anthony J.F. D'Apic al.

Serial No.: 08/984,900

Filed: December 4, 1997

Page

: 4

prior to disruption, encodes the porcine  $\alpha$ -1,3 galactosyltransferase with an amino acid sequence of SEQ ID NO:10.

Docket No.: 06868-005002

- 70. The porcine cell of claim 67, wherein said disruption is within exon 4, exon 7, exon 8, or exon 9 of the porcine  $\alpha$ -1,3 galactosyltransferase gene.
- 71. The porcine cell of claim 67, wherein said exogenous sequence is a selectable marker.
- 72. The porcine cell of claim 71, wherein said selectable marker is selected from the group consisting of the neo<sup>R</sup> gene and the hyg<sup>R</sup> gene.
- 73. The porcine cell of claim 67, wherein said exogenous sequence is flanked at its 5' and 3' ends by FRT DNA elements, and wherein stop codons have been inserted 3' to the selectable marker for each of the three reading frames for the porcine  $\alpha$ -1,3 galactosyltransferase gene.
- 74. The method of claim 51, wherein said disruption is within exon 4, exon 7, exon 8, or exon 9 of the porcine  $\alpha$ -1,3 galactosyltransferase gene.
  - 75. The method of claim 51, wherein said exogenous sequence is a selectable marker.
- 76. The method of claim 75, wherein said selectable marker is selected from the group consisting of the neo<sup>R</sup> gene and the hyg<sup>R</sup> gene.
- 77. The method of claim 51, wherein said exogenous sequence is flanked at its 5' and 3' ends by FRT DNA elements, and wherein stop codons have been inserted 3' to the selectable marker for each of the three reading frames for the porcine  $\alpha$ -1,3 galactosyltransferase gene.